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Toxicities of Copper, Zinc, and Cadmium Mixtures to Juvenile Chinook Salmon

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Abstract

Continual-flow toxicity tests were conducted to determine the acute toxic effects of copper, zinc, and cadmium mixtures on juvenile chinook salmon *Oncorhynchus tshawytscha*. Median lethal concentrations during 4 days (96-hour LC50 values) were most variable for zinc (39 to 122 μg/liter), less so for cadmium (0.6 to 1.6 μg/liter), and least variable for copper (26 to 34 μg/liter). Sensitivities of fish to metal mixtures also were variable. No synergism occurred among various mixtures of metals: two- and three-metal combinations had additive or antagonistic toxic effects. A decrease in the copper:zinc (from 1:3 to 1:12) and copper:cadmium (from 1:0.028 to 1:0.083) ratios decreased the toxicity of mixtures. Water-quality criteria for the protection of fish against mixtures of metals probably need to be developed on a site-specific and ratio-specific basis with repetitive toxicity tests.

The purposes of this study were to examine the toxicities of copper, zinc, and cadmium mixtures to an important anadromous salmonid, the chinook salmon *Oncorhynchus tshawytscha* and to relate joint effects of the metals to water-quality criteria which will protect the species from acid-mine wastes containing several metals. Further, this information will facilitate decisions about possible benefits of removing one or more of the metals from acid-mine wastes.

Although the separate toxicities of copper, zinc, and cadmium to aquatic organisms have been studied extensively, those of two- and three-metal mixtures have been investigated only cursorily. Toxicities of metals in a mixture produce an integrated response in test organisms that can be quantified as an interaction. Marking and Mauck (1975) and Marking (1977) termed these interactions as greater than additive ("synergistic"), "additive," or less than additive ("antagonistic") toxicity. The basis of this system is completely additive toxicity, a condition in which the toxicity of a mixture is exactly equal to that predicted from its component toxicities. If the mixture is more toxic than the components, the interaction is synergistic, and if the mixture is less toxic than the components, the interaction is antagonistic. The concepts (quantities) of synergistic, additive, and antagonistic toxicity are important to scientists modeling the effects and developing criteria for multiple toxicants in the aquatic environment.

The discharge of acid-mine wastes into waters inhabited by salmonids is common in California (Wilson et al. 1981). The Spring Creek drainage of the Sacramento River receives the largest volume of mine waste, affecting the greatest downstream area, in California. Sporadic copper-removal treatment and rainfall cause the Cu:Zn:Cd ratio to vary between 1:3:0.02 and 1:12:0.08 (Finlayson and Wilson 1979). The Zn:Cd ratio of the waste remains essentially unchanged between 1:0.007 and 1:0.008. Previous investigations (Finlayson and Ashuckian 1979; Finlayson and Verrue 1980) have defined the chronic (60- to 83-day) toxicities of both the acid-mine waste and formulated copper-zinc mixtures to eggs, alevins, and swim-up fry of chinook salmon and steelhead *Salmo gairdneri*; from these studies, "safe" copper and zinc concentrations were estimated. These studies indicated that the interaction of copper and zinc in mixtures of higher Cu:Zn ratios were additive, but less than additive at lower ratios. Here, we confirm those findings for juvenile chinook salmon, a species native to the Sacramento River drainage.

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Methods

The juvenile chinook salmon used in the 1979 and 1980 tests were reared from eggs taken (one day each year) from adults at the California Department of Fish and Game's Nimbus Hatchery located on the American River, a tributary of the Sacramento River. The eggs and alevins were held in fiberglass vertical-flow incubators, and the resulting fry were transferred to stainless steel troughs. The eggs, alevins, and fry were subjected to normal cultural practices as outlined by Leitriz and Lewis (1979). The fish were transported from the hatchery 96 hours before the tests began to the Department's Water Pollution Control Laboratory 100 m away, and kept in 1,000-liter circular tanks with an adequate supply of flowing water. The fish were fed commercial salmon food while in the hatchery but were not fed for 96 hours prior to or during the toxicity tests.

Sand-filtered water from the American River used for the May-July tests was consistent between years: pH 7.1–7.2; 18–19 mg/liter $CaCO_3$ alkalinity; 33–40 mg/liter dissolved solids; 20–21 mg/liter $CaCO_3$ hardness; less-than-detectable concentrations of most toxic metals; temperature 11–13 C; dissolved oxygen above 85% saturation.

All tests were conducted in 15-liter plexiglass troughs with the water volume adjusted to 12 liters; 50% water replacement occurred in 2.5 hours. The troughs were dosed by proportional diluters (Mount and Brungs 1967) and pre-dilution systems (Finlayson and Ashuckian 1979). Two to five tests were conducted simultaneously.

The fish were challenged for 96 hours with solutions of copper, zinc, and cadmium individually, of copper–zinc mixtures (ratios of 1:3 and 1:12), of copper–cadmium mixtures (ratios of 1:0.028 and 1:0.083), of a zinc–cadmium mixture (ratio of 1:0.008), and of copper–zinc–cadmium mixtures (ratios of 1:3:0.02 and 1:12:0.08). The solutions were prepared from reagent grade $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, and $3CdSO_4 \cdot 8H_2O$ in deionized water acidified to pH 1.8 with H_2SO_4. All stock solutions were checked analytically.

Twenty fish per trough (40 fish per concentration) was the stocking density for all tests. Mean fish fork length ranged from 3.9 to 6.8 cm. The loading factors ranged from 1.8 to 5.8 g/liter of trough volume and from 0.03 to 0.08 g/liter·day based on the flow rate through the troughs. These factors were within the recommended limits for continual-flow toxicity tests (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975).

Before each test, the troughs were rinsed for 48 hours with dilution water, which decreased metal concentrations from the prior test to <2 μg/liter Cu and Zn, and <0.2 μg/liter Cd. The new test solutions were added to the troughs 24 hours before the next test; metal concentrations approached nominal within 12 hours. Fluorescent lighting was on between 0700 and 1700 hours. Troughs were observed and sampled between 1000 and 1400 hours daily. Dissolved oxygen, temperature, and pH were each measured in each trough three times, and hardness and alkalinity once during the tests. Water samples from each trough were collected 24 and 72 hours following the start of each test and analyzed for concentrations of dissolved copper, zinc, and cadmium. Water samples (15 ml) were collected in a SESI® nylon syringe and filtered through a Gelman® type AE 25-mm glass fiber filter (porosity of 0.3 μm) held in a Nuclepore® 25-mm plastic valve. The syringe, filter, and valve were rinsed with 1 N HNO_3 and then rinsed twice with deionized water before each sample was taken. The samples were deposited in metal-free Nalgene® 30-ml linear polyethylene (LPE) narrow-mouth bottles and preserved with 0.3 ml of 6 N HNO_3. Water samples (30 ml) also were taken occasionally from the troughs for analyses of total metal concentrations in LPE bottles that were opened, filled, and closed while submerged; these water samples were preserved with 0.5 ml of 6 N HNO_3.

Copper, zinc, and cadmium concentrations were determined by air–acetylene or graphite-furnace atomic absorption spectrophotometry, depending on the metal and concentration. The detection limits were 2 μg/liter for copper and zinc, and 0.2 μg/liter for cadmium. Analytical precisions (2σ) were determined from the Shewhart (1931) equation

$$\sigma = \sqrt{(x - \bar{x})^2 / N - 1},$$

where $x = (A_1 - A_2)/(A_1 + A_2)$, $\bar{x}$ is the mean, A_1 and A_2 are paired observations, and N is number of observations. Precisions were ±4.4%

for copper, ±6.7% for zinc, and ±12% for cadmium.

Log-probit (solution concentrations versus mortality) analyses (Finley 1971) were used to calculate the LC50 (concentration causing 50% mortality) and LC10 (10% mortality) values by maximum-likelihood curve-fitting. Mortalities (M) in the test troughs were corrected for control mortality by Abbott's (Finley 1971) correction factor

$$M = 1 - Sx/Sc,$$

where Sx is survival in concentration x, and Sc is survival in controls. Copper, zinc, and cadmium concentrations at the LC50 and LC10 levels were interpolated from least-squares regressions (solution concentrations versus metal concentrations).

Toxicities of the metals in mixtures were expressed as toxic units (1 toxic unit of a metal = 96-hour LC50 concentration of that metal) and summed (Lloyd 1961; Brown 1968):

$$(Am/Ai) + (Bm/Bi) + (Cm/Ci) = S \qquad (1)$$

where A, B, and C are metals, i is the LC50 of an individual metal tested separately, and m is the concentration of an individual metal at the mixture LC50 value (Marking 1977). When $S = 1$, the toxicities of individual metals are additive in the mixture. When $S < 1$, the toxic interaction is synergistic: the metals are more potent in the mixture than they are separately. When $S > 1$, the toxic interaction is antagonistic: the metals are less potent in the mixture than they are separately. The potential range of S is $0-\infty$. To correct the asymmetry of S about 1.0, Marking (1977) suggested an additive index (AI), calculated as follows:

$$\text{if } S \geq 1.0, \text{ then } AI = -S + 1.0; \qquad (2)$$
$$\text{if } S \leq 1.0, \text{ then } AI = (1/S) - 1.0. \qquad (3)$$

This index is symmetrical about AI = 0 (additivity): positive values indicate synergism, negative values antagonism.

The statistical significance of the AI for the two- and three-metal mixture tests was estimated by substituting confidence intervals for the 96-hour LC50 values into Equations (2) and (3) and establishing a range (Marking 1977). The statistical significance of completely additive toxicity (AI = 0) for the two- and three-metal mixture tests was estimated by adjusting the metal mixture LC50 values to $S = 1.0$, substituting the adjusted confidence intervals into the two equations and establishing a range. Because LC50 values for the separate metals and metal mixtures were variable, and 95% confidence intervals sometimes overlapped zero, we used 75% confidence intervals for the analyses so as to detect tendencies of potential importance.

Results and Discussion

Mean water temperatures in the troughs were 11–13 C, and varied less than 0.5 C among troughs during concurrent tests. Trough pH varied from 7.3 in controls to 7.0 at the highest metal concentrations, hardness was 20–22 mg/liter as $CaCO_3$, and dissolved oxygen averaged 90% of saturation.

Mean dissolved metal concentrations in the control troughs were below detection (ranges: <2–3 μg/liter Cu; <2–5 μg/liter Zn; <0.2–0.3 μg/liter Cd). Measured total metal concentrations averaged 95 ± 15% (±SD) of expected. Dissolved metal concentrations averaged 79.3 ± 12.0% for copper, 90.6 ± 7.0% for zinc, and 87.1 ± 6.9% for cadmium of expected concentrations.

The 96-hour LC50 values (Table 1) were least variable for copper, more so for cadmium, and most variable for zinc. Neither fish size nor test year affected the sensitivity of chinook salmon to the metals. Our 96-hour LC50 values agree with those of Chapman (1978) for chinook salmon swim-up fry in water of similar quality, even though our fish were 1 to 3 months older. Chapman (1978) found fish sensitivity to all three metals (especially zinc) decreased from the swim-up fry stage to the parr stage, but the age range of our fish was too narrow for such a trend to be detected.

The two- and three-metal mixtures had variable toxicities (Table 1). Copper–zinc mixtures produced AI ranges indicating antagonistic toxicity: all AI values were negative and few overlapped the additive ranges (Fig. 1). The lower Cu:Zn ratio (1:12) was more antagonistic than the higher (1:3). These results conflict with some previous work. For example, our analysis of Sprague's (1964) data produced an AI value of 1.08 (synergism) for a Cu:Zn ratio of 1:11 applied to juvenile Atlantic salmon *Salmo salar* in soft water. Our analysis of additional studies by Sprague and Ramsey (1965) on juvenile At-

TABLE 1.—*Mean (±75% [95% in parentheses] confidence interval) concentrations (µg/liter) of copper, zinc, and cadmium causing 50% (LC50) and 10% (LC10) mortality of juvenile chinook salmon in 96 hours.*

		Copper		Zinc		Cadmium	
Solution	*N*	LC50	LC10	LC50	LC10	LC50	LC10
Cu alone	4	32 ± 4 (6)	19 ± 3 (6)				
Zn alone	4			84 ± 48 (81)	40 ± 24 (41)		
Cd alone	4					1.1 ± 0.4 (0.7)	0.8 ± 0.3 (0.6)
Cu:Zn = 1:3	4	33 ± 6 (10)	22 ± 3 (5)	102 ± 22 (33)	67 ± 18 (30)		
Cu:Zn = 1:12	4	21 ± 7 (12)	12 ± 7 (11)	263 ± 95 (163)	164 ± 90 (154)		
Cu:Cd = 1:0.028	3	23 ± 4 (7)	9 ± 3 (6)			0.8 ± 0.3 (0.6)	0.4 ± 0.2 (0.3)
Cu:Cd = 1:0.083	2	12 ± 3 (6)	6 ± 2 (3)			1.2 ± 0.4 (0.7)	0.4 ± 0.1 (0.1)
Zn:Cd = 1:0.008	3			87 ± 55 (94)	37 ± 26 (49)	0.7 ± 0.3 (0.5)	0.3 ± 0.1 (0.2)
Cu:Zn:Cd = 1:3:0.02	5	37 ± 14 (24)	26 ± 13 (21)	121 ± 39 (87)	82 ± 41 (70)	1.1 ± 0.5 (0.8)	0.8 ± 0.4 (0.7)
Cu:Zn:Cd = 1:12:0.08	5	18 ± 4 (7)	12 ± 5 (8)	218 ± 51 (86)	133 ± 53 (120)	1.8 ± 0.3 (0.6)	1.0 ± 0.5 (0.9)

lantic salmon produced an AI value of 0.00 (additive toxicity) in soft water at a Cu:Zn ratio of 1:15 with a test duration of 168 hours. Additionally, Lloyd (1961) found additive toxicity at a Cu:Zn ratio of 1:16 in a 7-day test with rainbow trout *Salmo gairdneri* in soft water. Conversely, unpublished data of Thompson et al. (1969; see References for source) produced an AI value of −1.61 indicating antagonism for juvenile steelheads. Their fish came, as did ours, from the Nimbus Hatchery and the tests were conducted in water of similar quality, so their results and ours are directly comparable. Additionally, the 96-hour LC50 values for chinook salmon fry exposed to Cu:Zn ratios of 1:2 and 1:11 reported by Finlayson and Verrue (1980) are reasonably close to those for the 1:3 and 1:12 ratios.

Copper–cadmium mixtures produced AI values indicating additive toxicity; test and additive ranges broadly overlapped (Fig. 1). The higher Cu:Cd ratio (1:0.028) was more additive than the lower (1:0.083). The zinc–cadmium mixture (1:0.008 ratio) had additive toxicity because a greater proportion of the AI test range was in the additive range rather than the antagonistic range. No published studies on toxicities of copper–cadmium or zinc–cadmium mixtures are available for comparison with our results.

Tests with two-metal mixtures indicated that changes in the amount of copper relative to both zinc and cadmium would change the type of interaction in copper–zinc–cadmium mixtures. In such three-metal mixtures, a tendency towards antagonistic toxicity would be expected at the lower Cu:Zn:Cd ratio (1:12:0.08); this was borne out by our tests (Fig. 1). Copper–zinc–cadmium mixtures produced AI ranges indicating antagonistic toxicity, and the lower copper ratio (1:12:0.08) was more antagonistic than the higher (1:3:0.02). The nonadditive toxicity of mixtures of these three metals is contrary to the data of Eaton (1973), which indicate an AI value of 0.26 (our analysis) in 96-hour tests with fathead minnows *Pimephales promelas* in hard water at a Cu:Zn:Cd ratio of 1:10:2. No comparable studies with salmonids (specifically chinook salmon) in soft water are available. Even if they were, comparisons of our tests with other work suggest that such analyses may be valid only on a site-specific and ratio-specific basis.

Warren (1971) proposed the term "no-interaction" (independent action) in the range of antagonistic toxicity when the toxicity of a mixture is determined solely by the toxicant in the greatest proportion. On the AI scale, independent toxic action should approximate the values of −1 and −2 for two- and three-metal mixtures, respectively. Thus, the mixtures which were not strongly additive (Cu:Cd ratio of 1:0.083 and Zn:Cd ratio of 1:0.008) or antagonistic (Cu:Zn ratio of 1:3 and Cu:Zn:Cd ratio of 1:3:0.02) could be exhibiting independent toxic action. Regardless of the terms employed, additive toxicity was not present in the three-metal mixtures and, should not be factored into deriving criteria for these metals in the Sacramento River.

Because our tests indicated antagonistic (or independent) toxicity for the three-metal mixtures, safe metal concentrations for chinook salmon that are estimated from tests of the individual metals should be suitable for mixtures as well. Chapman (1978) determined swim-up fry of chinook salmon to be the species' most

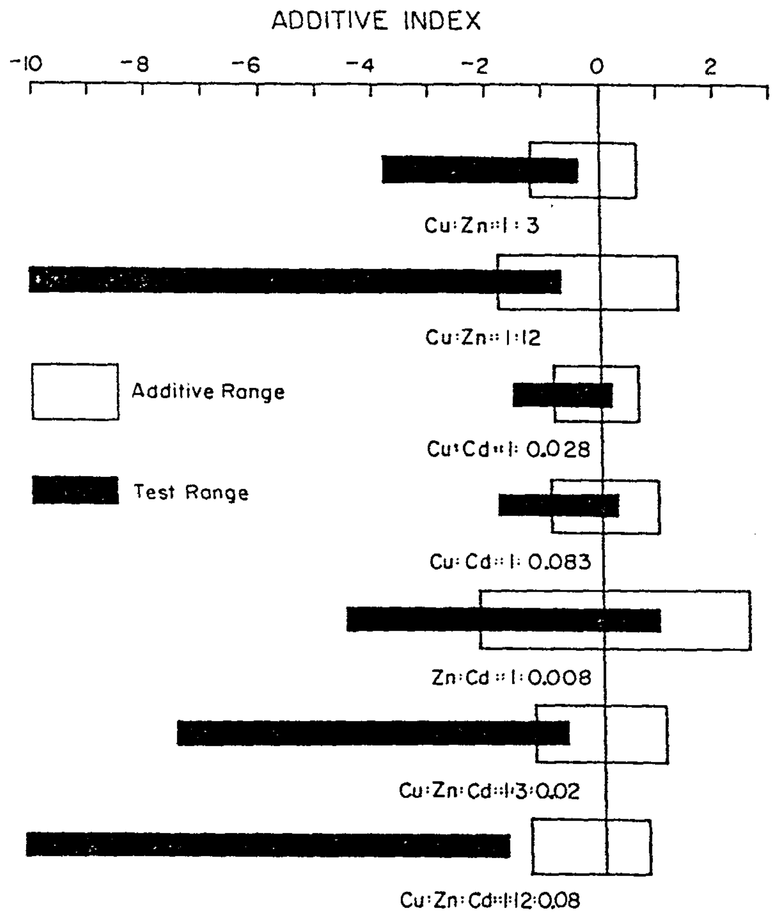

FIGURE 1.—*Additive indexes of two- and three-metal mixtures of copper, zinc, and cadmium in toxicity tests with juvenile chinook salmon (test ranges) compared with calculated ranges of simple metal additivity. Index ranges are based on 75% confidence intervals.*

sensitive life-history stage, so safe concentrations based on this stage should protect all stages. For the individual metals, our mean 96-hour LC10 values approximate Chapman's 210-hour LC10 values (in μg/liter): 19 versus 14 for Cu; 40 versus 60 for Zn; 0.8 versus 1.2 for Cd. Recommended safe levels for zinc (50 μg/liter total Zn) developed by McKim et al. (1979) and for cadmium (0.4 to 0.8 μg/liter dissolved Cd) developed by Davies et al. (1979) for relatively soft waters (up to 50 mg/liter $CaCO_3$) approximate our LC10 values and, therefore, are too high to be safe for chinook salmon. The recommended safe level for copper (0.1 times 96-hour LC50) developed by Windom et al. (1979) can be applied here.

Safe concentrations for the metals can be derived from the three-metal mixture tests. The mean metal concentrations at the 96-hour LC50 for Cu:Zn:Cd ratios of 1:3:0.02 and 1:12:0.08 were, respectively (in μg/liter), 37 and 18 for Cu, 121 and 218 for Zn, and 1.1 and 1.8 for Cd. The safe copper concentrations (0.1 times 96-hour LC50) for these two mixtures would be 4 and 2 μg/liter, respectively. Dilution of the Cu:Zn:Cd ratio mixtures of 1:3:0.02 and 1:12:0.08 to these safe copper concentrations would yield safe concentrations of, respectively (in μg/liter), 12 and 22 for Zn, and 0.1 and 0.2 for Cd; these are below the safe concentrations recommended for the individual metals (McKim et al. 1979; Davies et al. 1979).

This study indicates that the selective removal of copper from acid-mine waste (copper–zinc–

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cadmium mixtures) discharges in California is effective in decreasing the toxicity of the waste. Decreasing the Cu:Zn:Cd ratio from 1:3:0.02 to 1:12:0.08 increased antagonistic toxicity. Based on our safe concentrations, a 50% reduction in copper concentration will allow for an 83% increase in zinc and a 100% increase in cadmium concentrations with no increase in toxicity to chinook salmon.

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